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# 6

Annual Reports :: Year 6 :: Michigan State University

Project Report: Genetics of Permafrost Bacteria

## Project Investigators:

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## Project Progress

A major goal of our research team is to identify genes that enable bacteria to inhabit the permafrost environment. To accomplish this, we need to develop genetic systems to manipulate (including mutagenize) the genomes of the permafrost isolates. Progress has been slow, but recent results are encouraging. Several factors that had been hampering transformation of *Psychrobacter* 273–4 have been identified. First, a methylation sensitive restriction system has been shown to destroy foreign deoxyribonucleic acid (DNA) that has been methylated. To overcome this, we now propagate plasmids for transformation in *Escherichia coli* GM2163 ( *dam* – *dcm* – ) which does not methylate critical sites recognized by the *Psychrobacter* 273–4 restriction system. Second, we have found that successful electroporation of *Psychrobacter* 273–4 requires high voltages of 20–25kV/cm (normal *E. coli* conditions are 10–12.5kV/cm) and long recovery times after electroporation (between 8–24 h). Third, antibiotic resistance markers for kanamycin and chloramphenicol have been found to be poorly expressed. But taking these factors into account, we can now transform *Psychrobacter* 273–4 by electroporation. In addition, we have identified conditions whereby we can introduce wide host range plasmids, including pRL412, an RSF1010, into *Psychrobacter* 273–4 by conjugation. Finally, we have examined natural competence of *Psychrobacter* 273–4 and *Psychrobacter cryopegella* . Transformation frequencies (using *Psychrobacter* DNA) of about  $10^{-6}$  to  $10^{-7}$  and  $10^{-8}$  to  $10^{-9}$  transformants per recipient per  $\mu\text{g}$  of DNA have been obtained with *Psychrobacter* 273–4 and *Psychrobacter cryopegella* , respectively. The bacteria are transformable in the stationary phase of growth if cultures are initially grown in tryptic soy broth and subsequently incubated with DNA on marine agar (other combinations of growth stage and media have been tried and have not yielded transformants).

## Highlights

- We can now introduce wide host range plasmids into *Psychrobacter* 273–4 by both electroporation and conjugation.

- A major barrier to transformation of plasmids into *Psychrobacter* 273–4 is a methylation sensitive restriction system.
- Conditions for natural transformation have been identified.

#### Roadmap Objectives

- **Objective No. 5.1:** Environment–dependent, molecular evolution in microorganisms
- **Objective No. 5.3:** Biochemical adaptation to extreme environments
- **Objective No. 6.2:** Adaptation and evolution of life beyond Earth